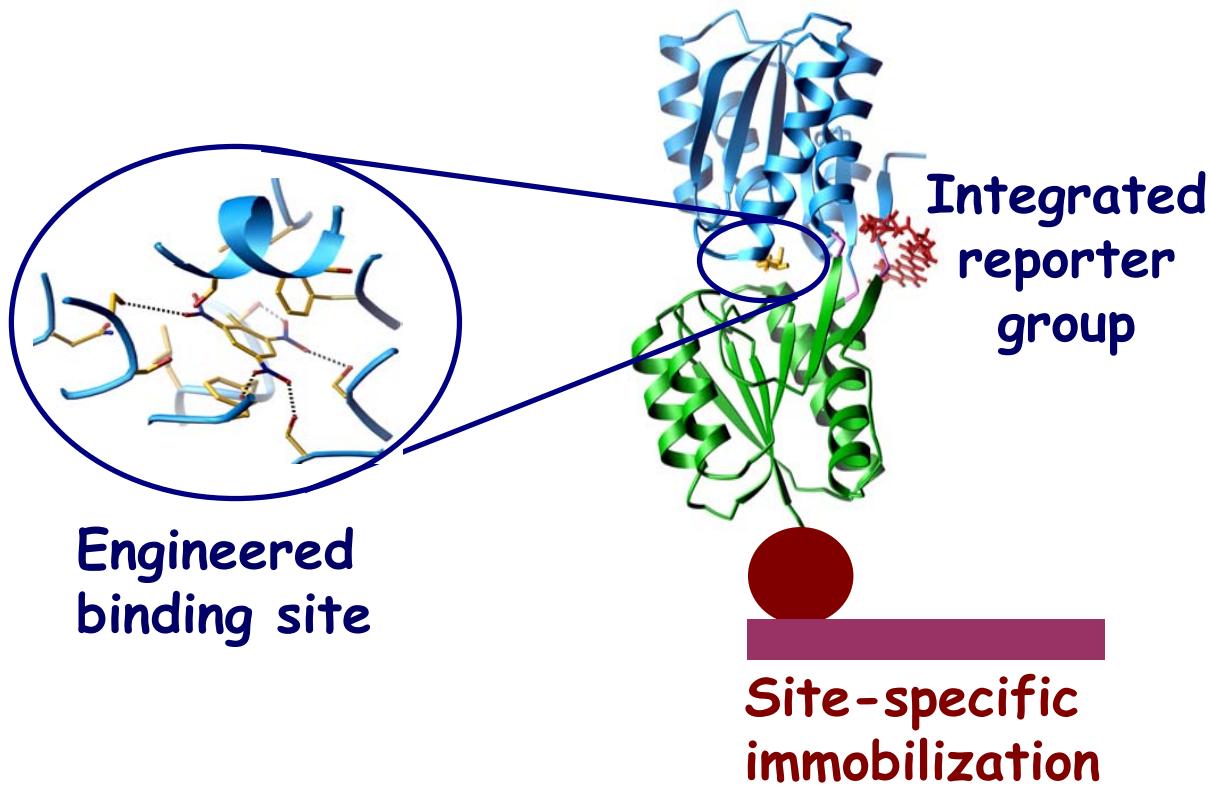
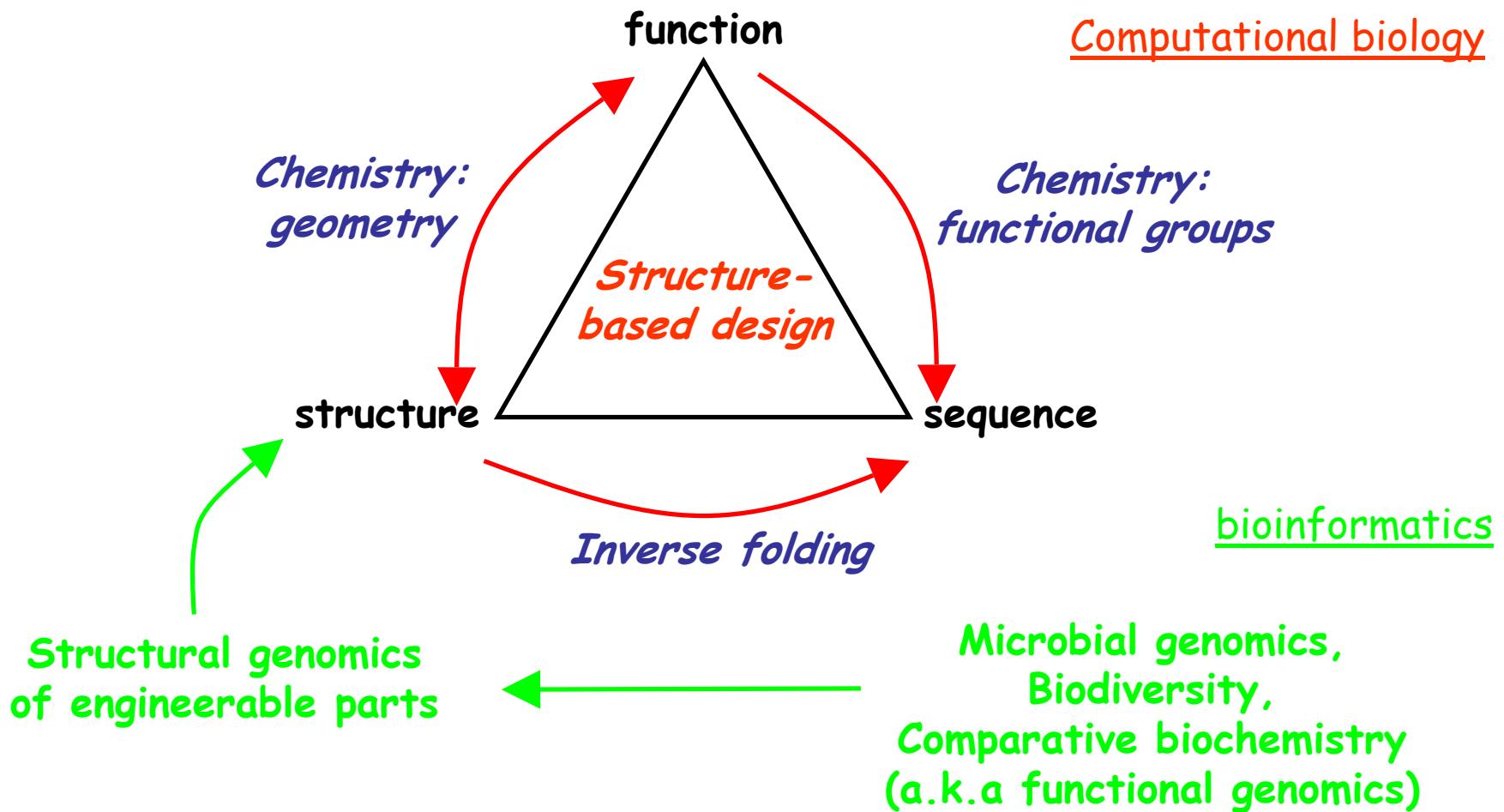


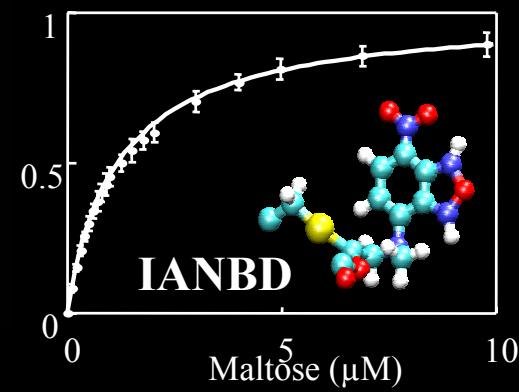
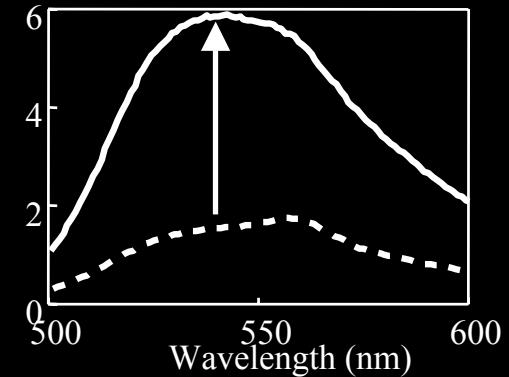
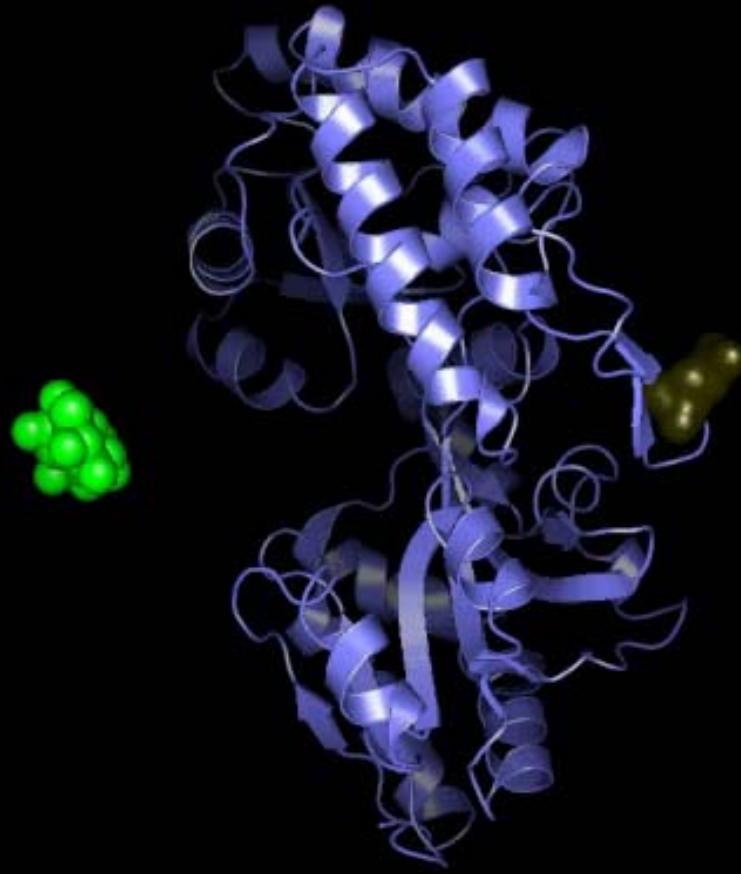
Modular design of reagentless biosensors for chemical defense



Computational Protein Design

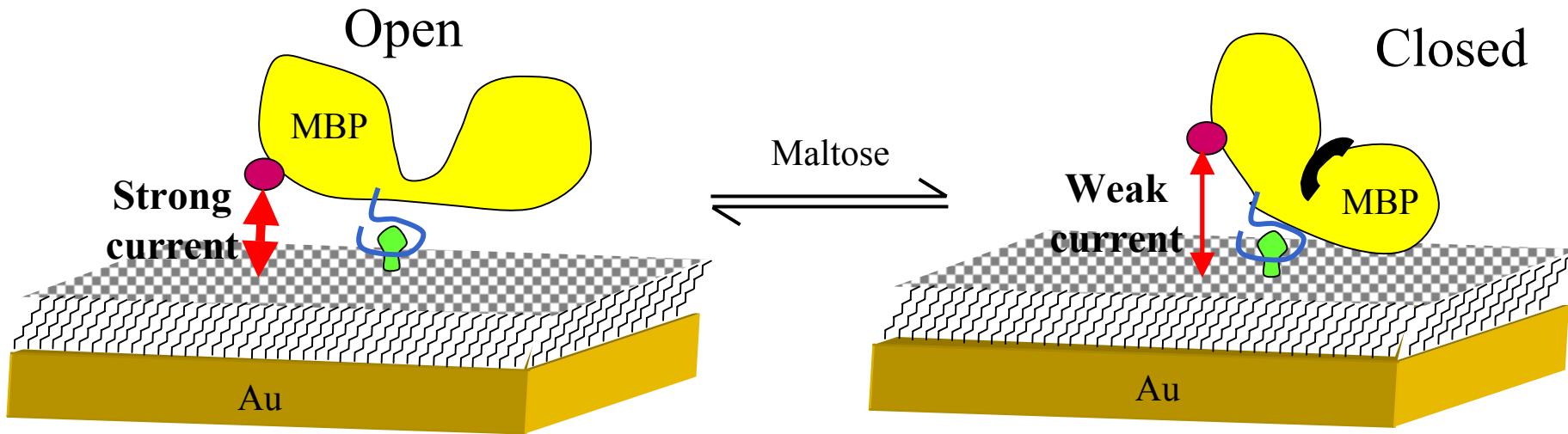


Fluorescent signal transduction by exploiting the hinge-bending mechanism in periplasmic-binding proteins

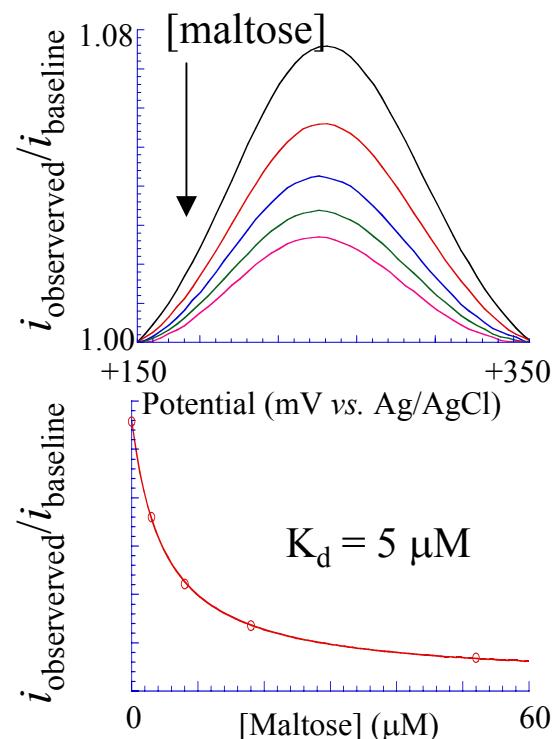
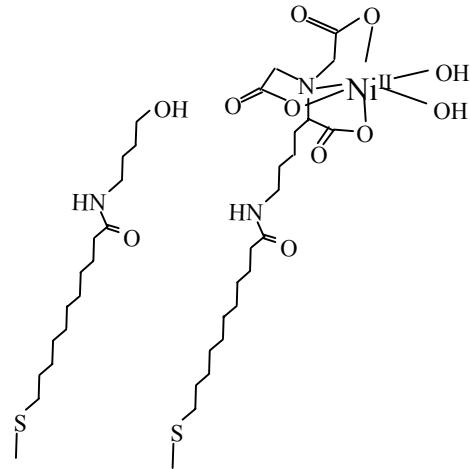


Marvin & Hellinga (1997) *Proc. Natl. Acad. Sci. USA*, 94:4366-4371.
Marvin & Hellinga (2001) *Nature Struct. Biol.*, 8:795-798

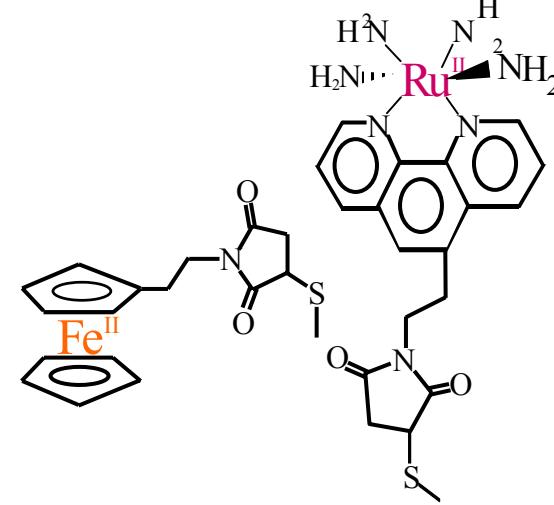
Electrochemical signal transduction



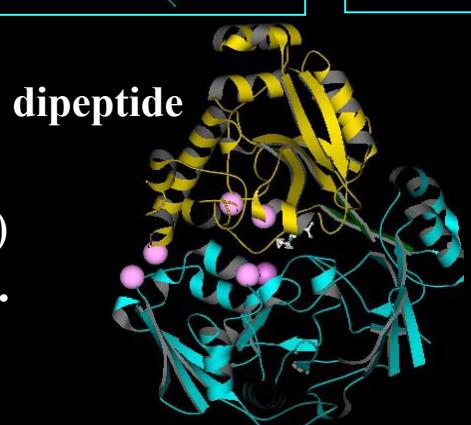
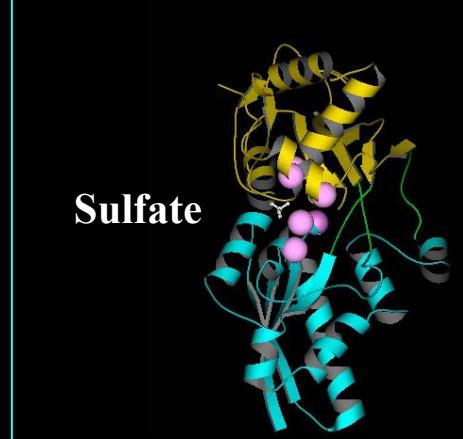
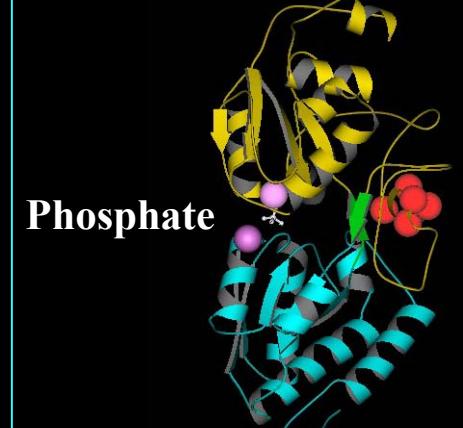
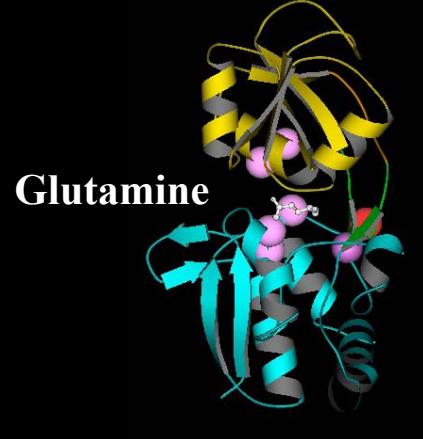
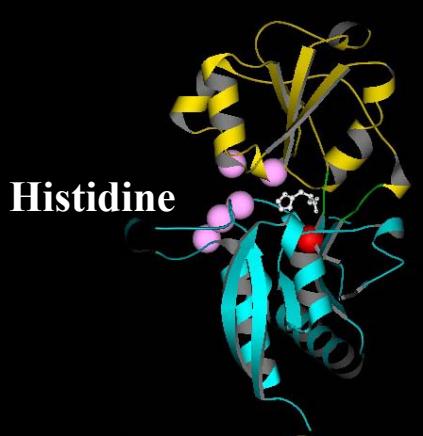
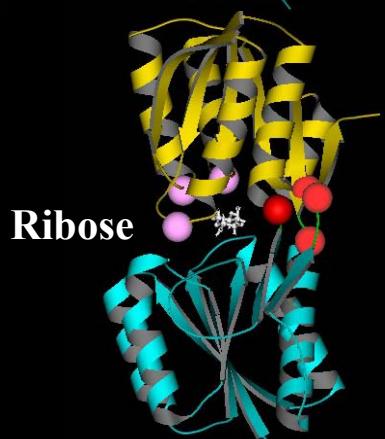
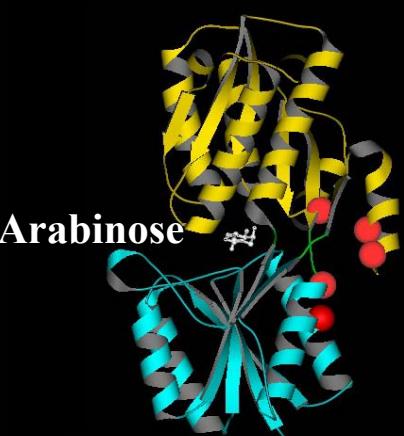
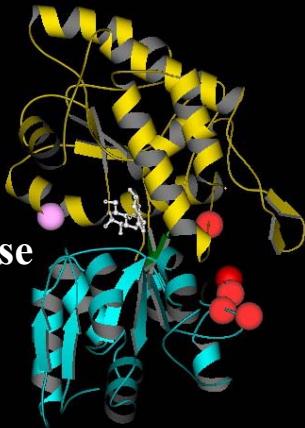
Monolayer Components:



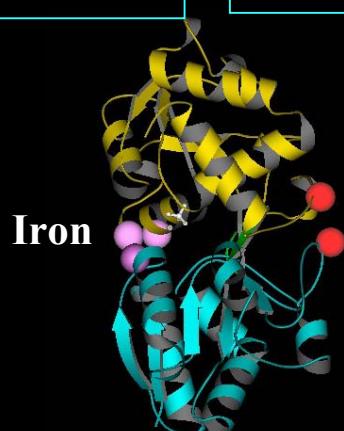
Electrochemical Reporters:



Trammell *et al.* (2001) *Bioconj. Chem.*, **12**:64



De Lorimier *et al.* (2002)
Prot. Sci., 11:2655-2675.



Protein

Maltose

MBP



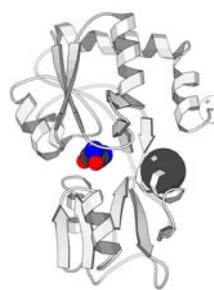
Glucose

GBP



Glutamine

QBP



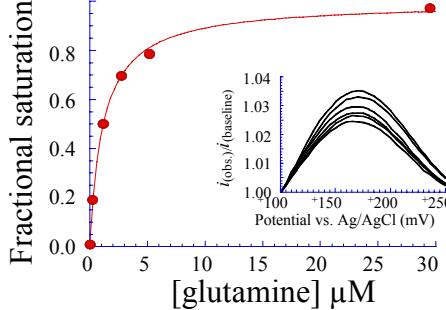
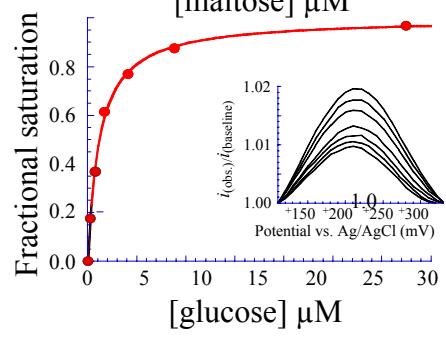
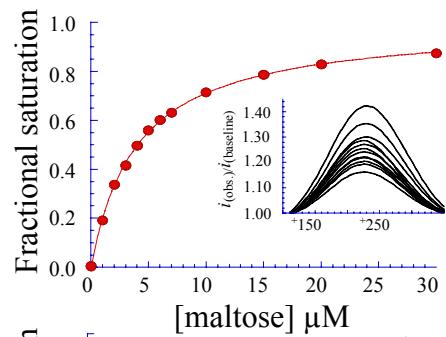
And all the others ...

Marvin & Hellinga (1998) *J. Am. Chem. Soc.*, **120**:7-11

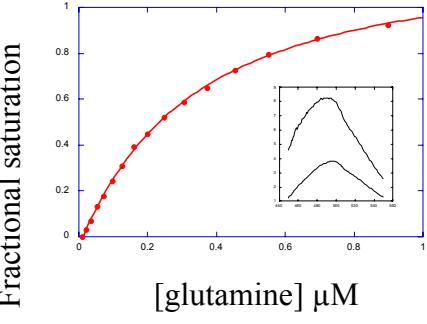
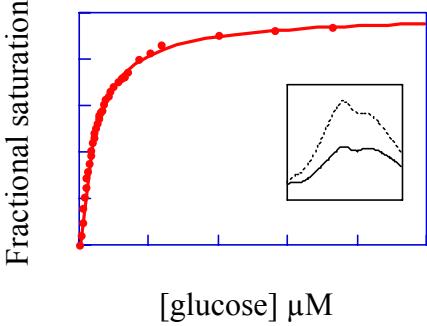
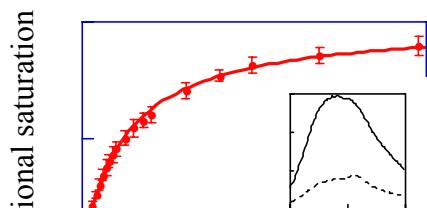
Marvin & Hellinga (2001) *Proc. Natl. Acad. Sci. USA*, **98**:4955-4960

DeLorimier et al. (2002) *Prot. Sci.*, **11**:2655-2675

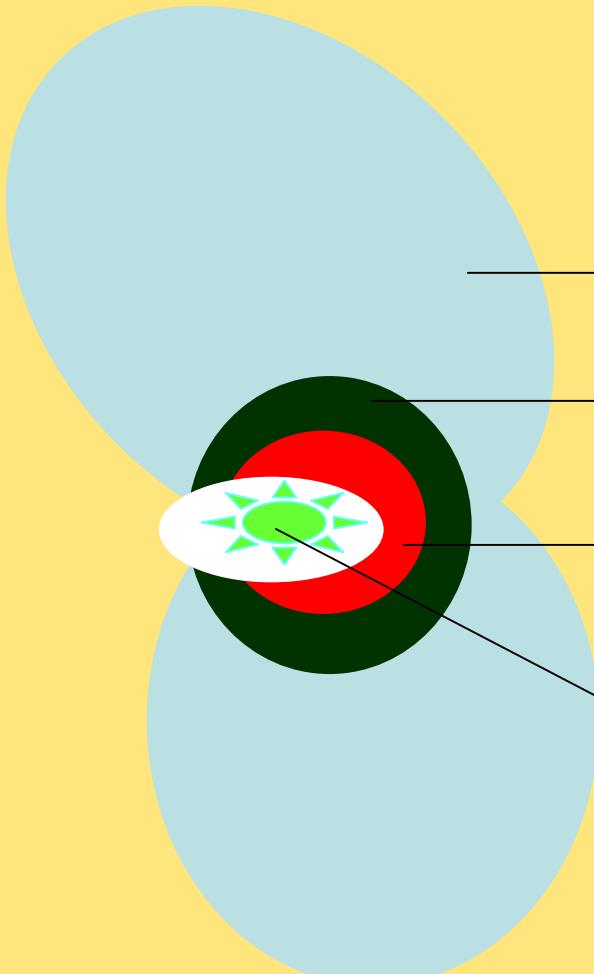
Bioelectronic sensing



Optical sensing



Computational design of ligand-binding sites



Conformation
Sequence

Minimize: $E(c,s) = \sum Vdw(s,c,r_{ij}) +$
 $\sum Elec(s,c,r_{ij}) +$
 $\sum Hb(s,c,r_{ij}) +$
 $\sum Solv(s,c,r_{ij}) +$
 $\sum S(s) +$
Hydrogen bond inventory

Molten zone :~30 residues

$6,122^{30}$ rotameric sequences

Evolving zone: ~12 residues

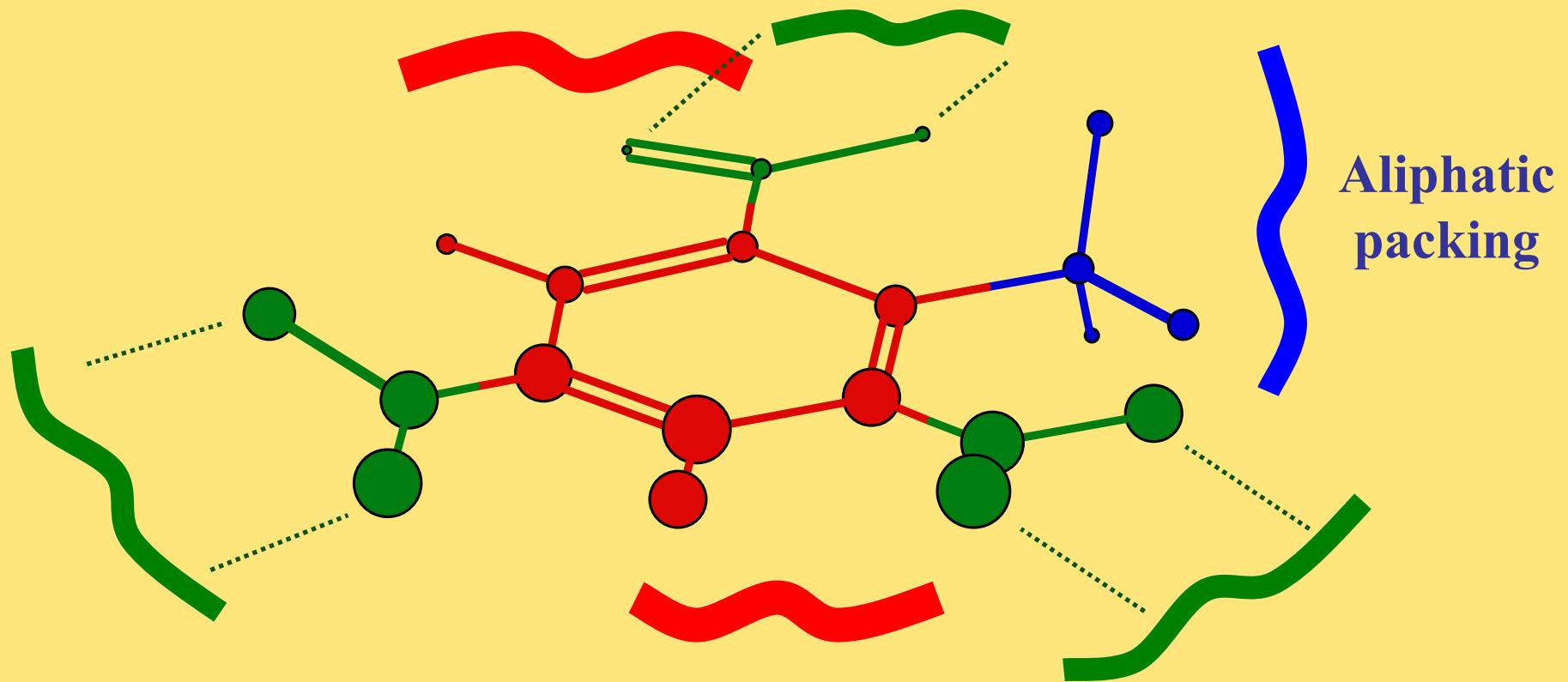
$6,122^{12}$ rotameric sequences

Docking zone: ligand with all rotational and translational degrees of freedom,
 10^6 - 10^8 poses

Total combinatorial complexity:
 10^{200} - 10^{1000} rotameric sequences

Computational design of 2,4,6-trinitrotoluene (TNT) receptors

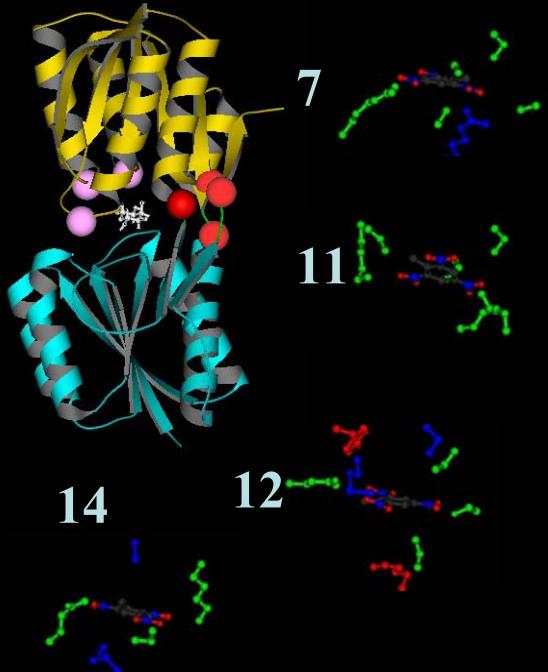
Aromatic stacking > Aliphatic packing



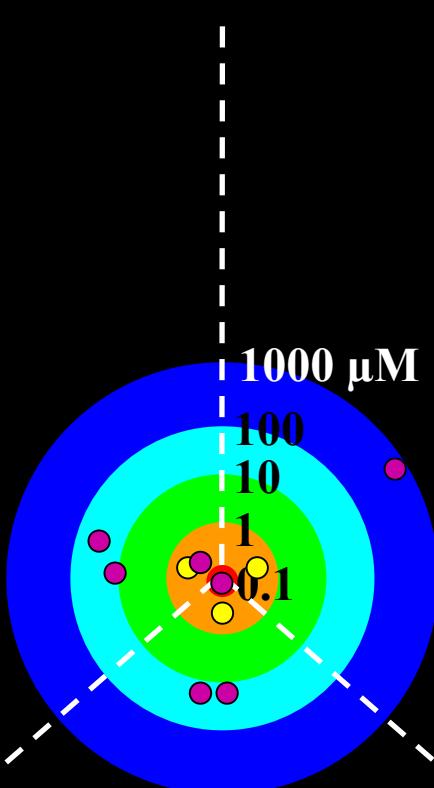
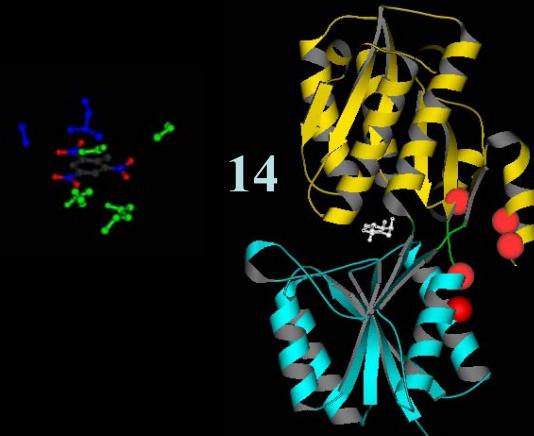
Hydrogen bonding:

Arg,Lys > Asn,Gln, Main-chain NH > Ser,Thr > water

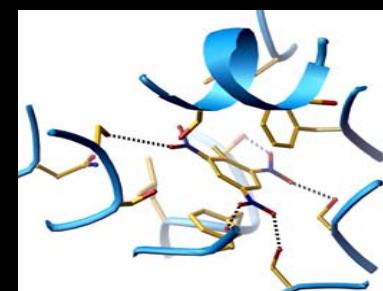
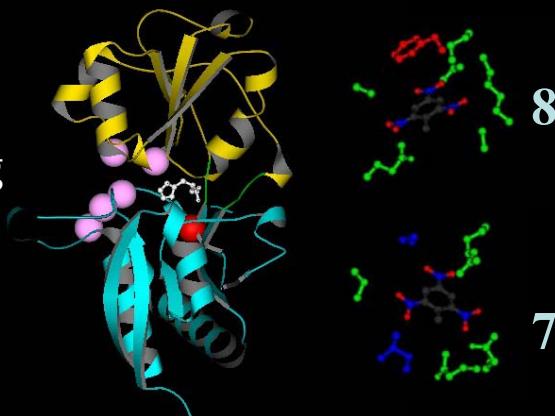
Ribose-binding protein
EZ = 17 residues



Arabinose-binding protein
EZ = 19 residues



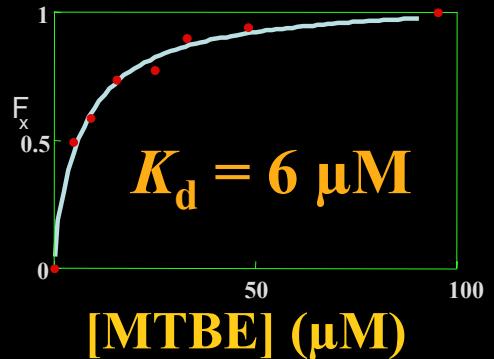
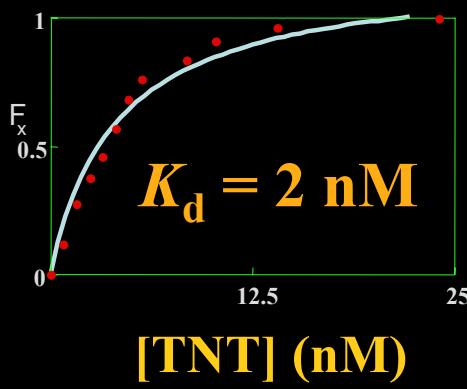
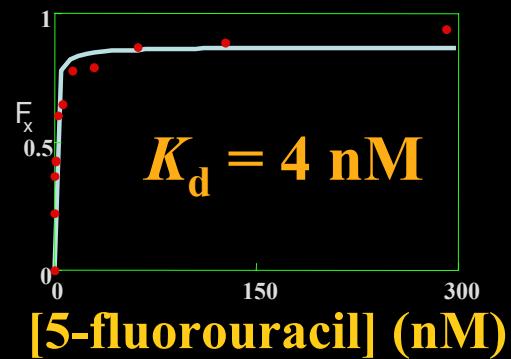
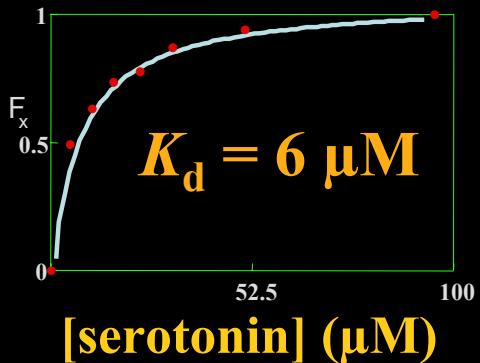
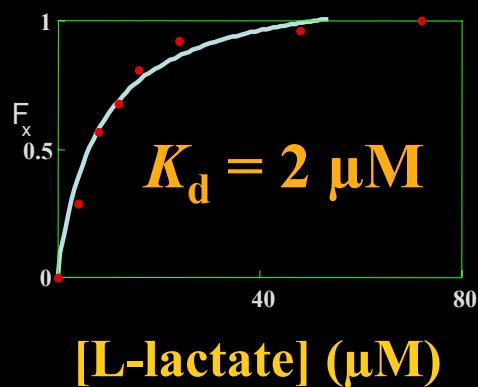
Histidine-binding
protein
EZ = 15 residues



Metabolites

Neurotransmitters

Drugs



Explosives

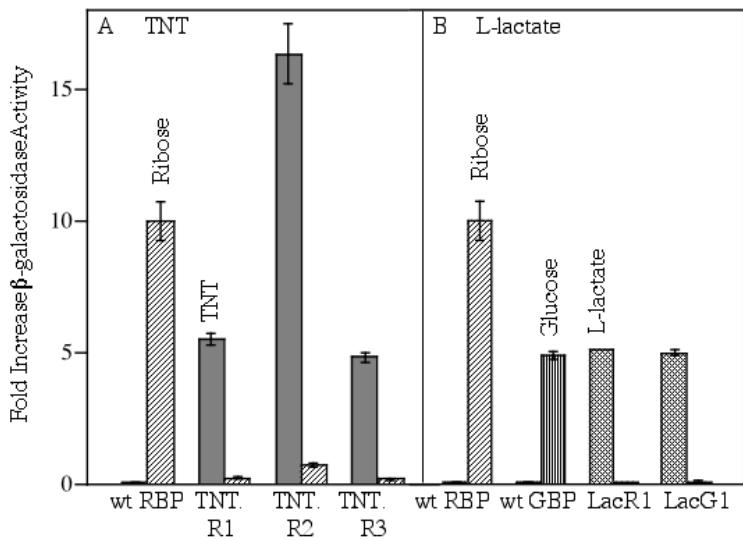
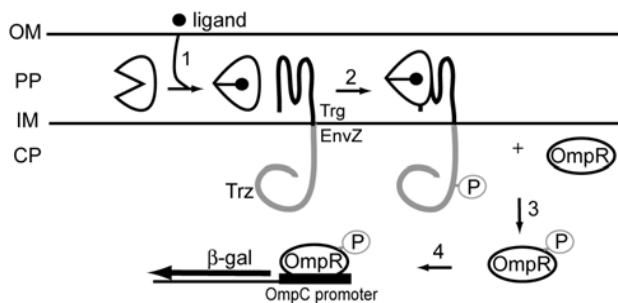


Pollutants

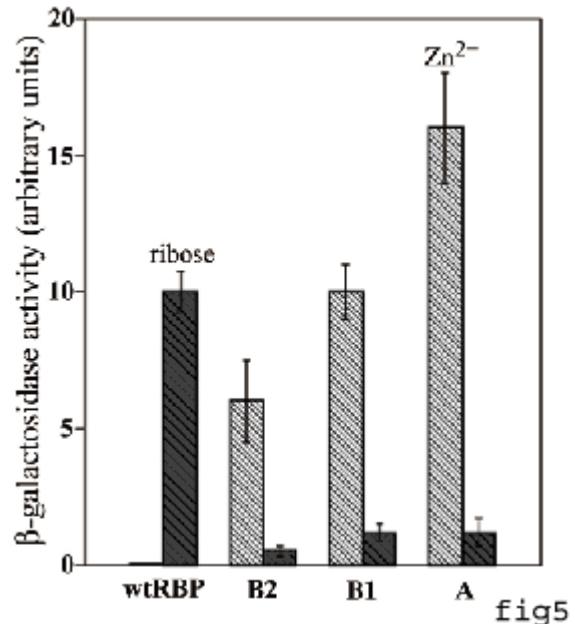


Chemical Threats

Signaling with designed receptors in the *trz* chimera

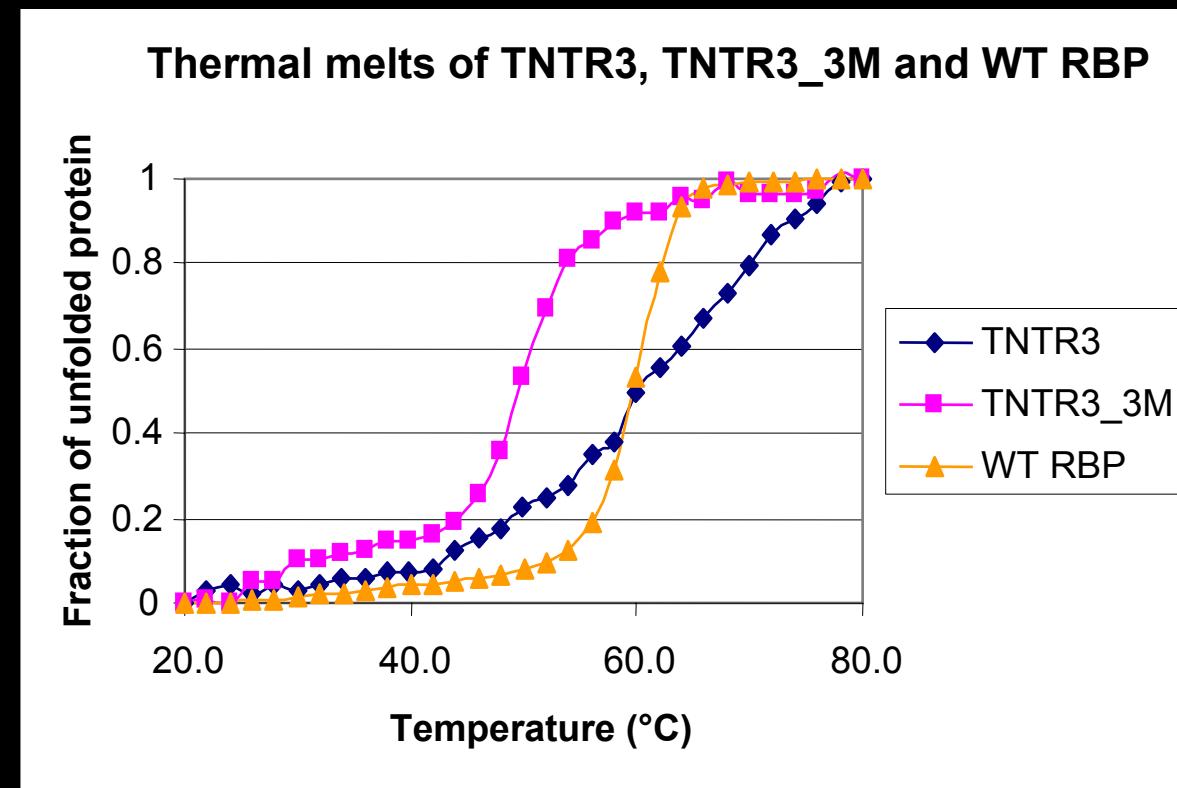
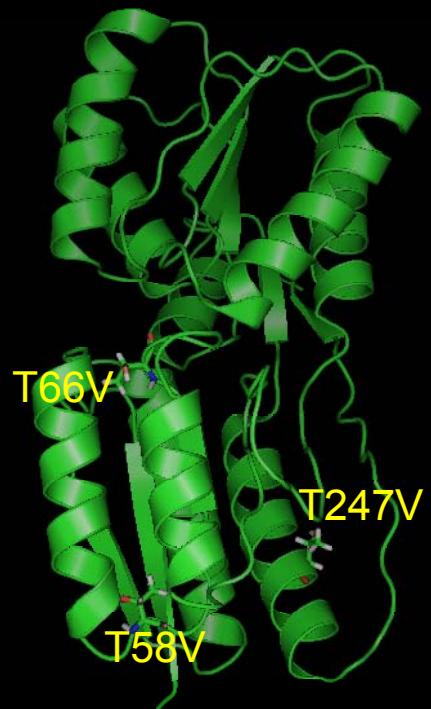


Looger, Dwyer, Smith, Hellinga
(2003) Nature, **423**:185.

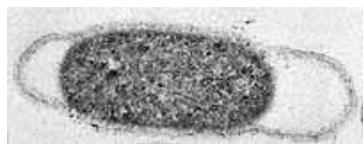
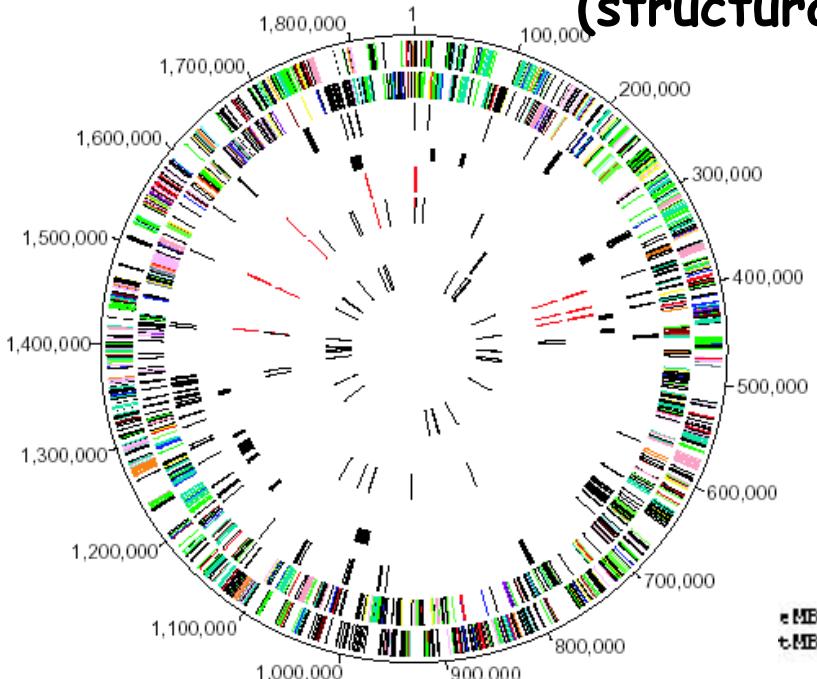


Dwyer, Looger, Hellinga (2003)
PNAS, **100**:11255

Rational mutations to increase thermal stability of TNTR3



Periplasmic binding proteins from the hyperthermophile *Thermotoga maritima* (structural genomics of engineerable parts)



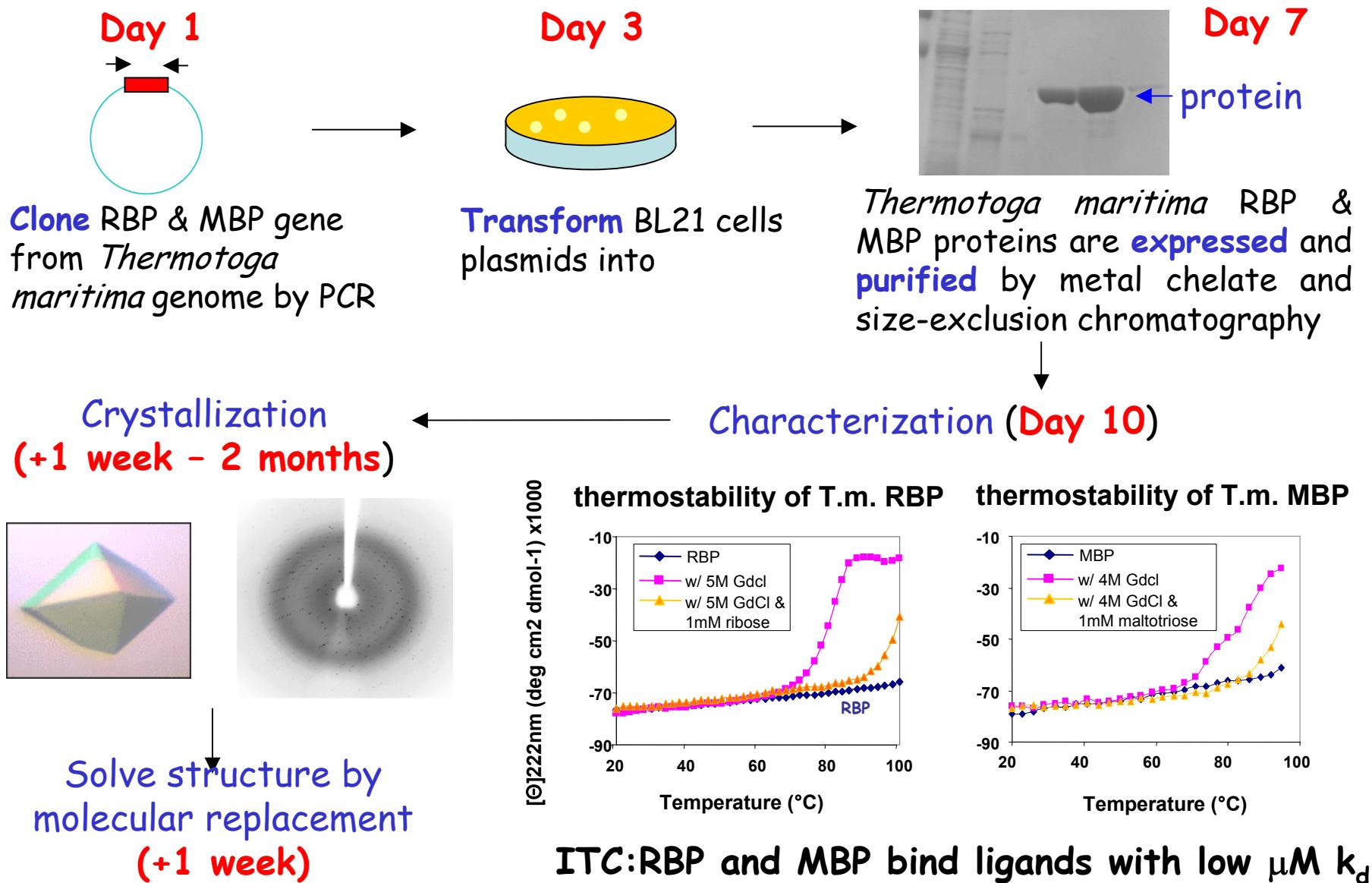
anaerobic bacterium from
marine mud on vulcano island, Italy
optimal growth at 80°C

TM1839 periplasmic **maltose**-Bprt
TM0958 periplasmic **ribose**-Bprt
TM0114 periplasmic **sugar**-Bprt
TM0810 periplasmic **sugar**-Bprt
TM0595 periplasmic **sugar**-Bprt
TM1855 periplasmic **sugar**-Bprt
TM0432 periplasmic **sugar**-Bprt
TM0418 periplasmic **sugar**-Bprt

(Nelson et al, 1999)

e-MBP	KIEEGKLV IWIWING DNG YN GLA EWF KKF EKIT GIKVT VEPDQK -- LEENFPQVAATGDPDI I FWDHDFGTY
t-MBP	MLS QTKLT IWC 3- ENQVD ILQQLG EEFKAKY GIPVEVQYVDFGS IK3 KFLTAAP QGQGADII VWAHDWQEL
	*** *** *
e-MBP	AQ3 GLLAE ITPIDKAFQDKLYPFTWIDAVRYNGKLIAYPIAVERAL3 LIYNKNDLLPNPPKTWEIIPALDKELKAK
t-MBP	AWNGLI EP IPNFSDILKN- FYDTALKAFPSYGGKLYGUPYAMAEVALIYNKIDFUDS VPKTMDELIEKAKQIDEE
	* *
e-MBP	GK --- 3 ALMFMNLQEPYFTWPL IAAAGC GYAFKYYEN GKYDI KDW GWINDA GAKAGLTLFLVIL IKNKHMRAIDTDY3
t-MBP	YGGEVRGF IYDVAMFVFS APP ILGYYG GYVFKETP QGLDWTDI GLANE GAWKGAKLIKEM IDE GWLTP GDNYG
	*** * * *** * * * * * * * * * * * * * * * * * * *
e-MBP	IAE&AFPK GET&MTINGGP&WJ3NIDT SKUNY GUTVLPPTFK-G QP SKPFW GWL 3A GINAA3 PPKELAKEFLNM
t-MBP	TMD SMFKE GLAAM I INGLMAIKSYMDAGI NYGVA API PELEP GWPAPKFW GWWQGFMINAK SPNNKVI AMEFLTN
	* * * * *** *
e-MBP	YLL TDEGL EA VNKNDKPLG AVALNS YEEELAKIDPR IAATMENAQK GEI MP NIP QM SAFQEA VRTAV INAA3 GR
t-MBP	FIARKETMYKIVYLADPR - LPARKDVL ELVKNDNPDVVAFT QS ASM GTPMP NWPEMAPQUSAMG DAL S I IINGQ
	* *
e-MBP	QTWDEALKD&QTR ITK ----
t-MBP	ANVEDA LNE2WEN IKA QI EKG

Cloning, characterization and crystallization of *Thermotoga maritima* RBP and MBP



Mary Dwyer
Shahir Rizk
Malin Allert
Matt Cuneo
Birte Hoecker
Yaji Tsien
Alex Miklos
Jeff Smith
David Conrad
Robert de Lorimier
Derek Jentz
Loren Looger
Shamil Shadikov
Jonathan Marvin
David Benson
Kevin Sali
Chad Paavola
Michael Wisz

biosensor design/sentinels/chiral separations/enzyme design
bio-nanomachines/biosensors
biosensors/enzyme design
sentinels/extremophiles/ppi design
enzyme design/extremophiles
engineered thermostability
biosensors/chiral separations
biosensors/sentinels
glucose sensing/bio-nanotechnology
biosensor design
receptor design/ppi design
computational design
molecular sensors/biomolecular interfaces
conformational coupling/fluorescent biosensors
metalloprotein design/bioelectronic sensors
glucose sensing protein
biosensor scaffolds
design of metalloenzymes/computational design

Funding

ONR, DARPA
NIH NSF
Whitaker Foundation

American Cancer Society
Juvenile Diabetes Foundation
Medtronics, LifeScan, Becton-Dickinson